

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Eva Altmann, et al.

Serial No.: 10/540,359

Filed: June 23, 2005

For: DERIVATIVES OF ARYL-QUINAZOLINE/ARYL-  
2-AMINO-PHENYL METHANONE WHICH  
PROMOTE THE RELEASE OF PARATHYROID  
HORMONE

Attorney Docket No.: PA/4-32832A

Group Art Unit: 1624

Examiner: Tamthom Ngo Truong

MS: Amendment

Commissioner for Patents

PO Box 1450

Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. LEO WIDLER

I, Leo Widler, a citizen of Switzerland, residing in Münchenstein/Switzerland, hereby declare as follows:

1. I received my PhD from the ETH (Federal Institute of Technology) located in Zürich/Switzerland, in 1983. After two years of post-doctoral studies at the Sandoz Research Institute in E. Hanover, NJ, USA I joined the pharmaceutical division of Ciba-Geigy as research chemist working for different projects in the areas of arthritis and bone metabolism. Shortly after the merger of Ciba and Sandoz (1997) I joined the so-called "calcilytics" project, first as coordinator of the chemistry efforts and in 2000 as the team leader of the whole project. I am currently employed by \_Novartis Pharmaceuticals AG, Basel, Switzerland, and I am a named inventor on the above-identified patent application. I have attached hereto a copy of my curriculum vitae (see, Appendix A)

which demonstrates that I am an expert in the field of medicinal chemistry and have particular knowledge and understanding in the areas of arthritis and bone metabolism.

2. The "calcilytics" project focused on the identification of compounds antagonizing the calcium-sensing receptor (CaSR) in the parathyroid gland: PTH secretion is controlled by a calcium-sensing receptor (CaSR), a member of the GPCR family 3, expressed on the surface of parathyroid cells. Extracellular  $\text{Ca}^{2+}$  concentrations below the normal physiological level stimulate PTH release, while at high  $\text{Ca}^{2+}$  concentrations little or no PTH is secreted. With a CaSR modulator, PTH levels can be controlled independently of extracellular  $\text{Ca}^{2+}$  concentrations. Calcimimetic compounds (agonists of CaSR) activate the receptor and inhibit PTH secretion, while calcilytics (antagonists of CaSR) mimic a state of hypocalcaemia by blocking the parathyroid cell CaSR located on the cell surface and thus stimulate the release of endogenous PTH which is stored in relatively large amounts in parathyroid cells. This PTH release results in transient increases in circulating PTH levels.

Since it is now well established that controlled treatment of patients with PTH and analogues thereof can have a pronounced anabolic effect on bone formation, compounds promoting PTH release, such as calcilytic compounds, may be used for preventing and treating conditions of bone which are associated with increased calcium depletion or resorption or in which stimulation of bone formation and calcium fixation in the bone is desirable, in particular osteoporosis.

However, it is well documented that elevated levels of PTH only result in higher bone mass if they are transient, i.e. they do not persist for more than about 2-4 hours. It has been found that a short period of elevation of PTH plasma levels is crucial for an effective therapeutic result, since constantly elevated plasma levels of PTH increase not only bone formation by activation of the osteoblasts, but also activate osteoclasts leading to an increase in bone resorption and result in a net loss of predominantly cortical bone. Therefore, successful calcilytic compounds have to show a pharmacokinetic (PK) profile inducing such a short period of increased PTH levels.

3. The instant application describes, in relevant parts, a new compound(s) (and salts thereof), pharmaceutical compositions comprising the compound(s) or salt thereof, and methods for treating osteoporosis, juvenile osteoporosis, menopausal osteoporosis, post-menopausal osteoporosis, post-traumatic osteoporosis, fractures,

osteopathy, osteo-malacia, periodontal bone loss or bone loss due to arthritis or osteoarthritis in which the new compound(s), or salt thereof, is administered to a patient. The compounds of the instant application are described as calcilytic compounds, i.e. compounds acting as antagonists at the parathyroid cell CaSR and as promoters of PTH release, therefore being useful in the treatment of conditions of bone which are associated with increased calcium depletion or resorption or in which stimulation of bone formation and calcium fixation in the bone is desirable, in particular the aforementioned conditions.

4. I have reviewed the Final Office Action issued by the Examiner on April 14, 2009 in the above-identified patent application, as well as co-pending U.S. Patent Application No. 10/480,559 cited therein. I understand that claims 11-13 and 23-25 in the above-identified patent application stand provisionally rejected, under the judicially created obviousness-type double patenting, as allegedly unpatentable over claims 25, 32 and 34 of co-pending U.S. patent application no. 10/480,559 (the '559 application). I understand that this means the Examiner believes that the compounds of claims 11-13 and 23-25 of the instant application are obvious given broad claims 25, 32 and 34 of the '559 application.

5. Comparative data provided herewith establishes that the species of claims 11 and 13 of the instant application have superior in vitro and in vivo potency, which properties are not found in, nor are they suggested by the examples of the '559 application. More particularly, comparative data is provided for the compound of claim 13 (Table 1), the structurally related, exemplified compounds recited in the '559 application (Table 2) and the most active exemplified compounds in the '559 application (Tables 3 and 4).

The comparative data describe the antagonistic potential of the compounds at the calcium-sensing receptor (CaSR) in the parathyroid gland either by the inhibition of intracellular calcium transients stimulated by extracellular calcium with a so-called FLIPR assay as described within the specification of the instant application in paragraph [0972] and as set out in more detail in paragraph 6 *infra*, and/or by the inhibition of calcium-induced inositol phosphate formation stimulated by extracellular calcium with a so-called PI assay as described within the specification of the instant application in paragraphs [0970] – [0971] and as set out in more detail in paragraph 7 *infra*. As indicated in

paragraph 2 *supra*, an optimal calcilytic must not only be a potent antagonist of the CaSR but, in addition, its pharmacokinetic (PK) profile must be appropriate in order to elicit a bone anabolic effect, i.e. it has to induce a rapid and short-lasting release of the PTH into the plasma. The required parameters for a good calcilytic are a high plasma concentration ( $C_{max}$ ) at an early time point ( $T_{max}$ ) and a short terminal half life to ensure expeditious return of exposure and PTH levels to baseline. Comparative PK data were determined with a standard assay for pharmacokinetic cassette dosing in rats as set out in more detail in paragraph 8 *infra*.

6. **Assay for intracellular free calcium (FLIPR assay).** Antagonism at the CaSR was determined by measuring the inhibition of intracellular calcium transients stimulated by extracellular calcium. CCL39 fibroblasts stably transfected with human PCaR (the gene coding for CaSR) were seeded at 40'000 cells /well into 96-well Viewplates and incubated for 24 hours. Medium was then removed and replaced with fresh medium containing 2  $\mu$ M Fluo-3 AM (Molecular Probes, Leiden, The Netherlands). In routine experiments, cells were incubated at 37°C, 5 % CO<sub>2</sub> for 1 h. Afterwards, plates were washed twice with mHBS and wells were refilled with 100  $\mu$ l mHBS containing the test compounds. Incubation was continued at room temperature for 15 minutes. To record changes of intracellular free calcium, plates were transferred to fluorescence-imaging plate reader (Molecular Devices, Sunnyvale, CA, USA). A baseline consisting in 5 measurements of 0.4 seconds each (laser excitation 488 nm) was recorded. Cells were then stimulated with calcium (2.5 mM final), and fluorescence changes recorded over a period of 3 minutes. Peak intracellular calcium levels were plotted as a function of test compound concentration, and IC<sub>50</sub> values were determined using the Origene curve fitting software.

7. **Assay for phosphoinositol formation (PI Assay).** To determine the antagonistic activity at the human parathyroid calcium-sensing receptor (CaSR), compounds were tested in functional assays measuring the inhibition of calcium-induced inositol phosphate formation in CCL39 fibroblasts stably transfected with human PCaR (the gene coding for CaSR). Cells were seeded into 24 well plates and grown to confluence. Cultures were then labeled with [<sup>3</sup>H]inositol (74 Mb/ml) in serum-free medium for 24h. After labeling, cells were washed once with a modified Hepes-buffered salt solution (mHBS: 130 mM NaCl, 5.4 mM KCl, 0.5 mM CaCl<sub>2</sub>, 0.9 mM MgSO<sub>4</sub>, 10 mM

glucose, 20 mM HEPES, pH 7.4) and incubated with mHBS at 37°C in the presence of 20 mM LiCl to block inositol monophosphatase activity. Test compounds were added 3 minutes before stimulating CaSR with 5.5 mM calcium and incubations continued for further 20 min. Thereafter, cells were extracted with 10 mM ice-cold formic acid and amounts of inositol phosphates formed were determined using anion exchange chromatography and liquid scintillation counting. Inositol phosphates levels were plotted as a function of test compound concentration to determine IC<sub>50</sub> values.

8. **Assay for pharmacokinetic (PK) cassette dosing in rats.** The experiment was performed in conscious, fed, permanently cannulated rats kept under standard conditions. Compounds were either administered as a cocktail of up to 7 compounds – so-called cassettes – including a well-characterized reference compound to check for drug-drug interactions within the cassette or as single compound in selected cases. The compound(s) were orally administered by gavage, at a concentration of 3 mg/kg in a microemulsion vehicle prepared by diluting a 1% w/w or 2% w/w solution of the respective compound(s) in a microemulsion preconcentrate (MEPC-A or MEPC-B) with deionized water to the desired final volume (typically 2.5 mL/kg total administration volume). The MEPCs had the following compositions:

	MEPC-A	MEPC-B
Cremophor® RH40	45% w/w	43% w/w
Corn oil glycerides	36% w/w	35.7% w/w
Tocopherol, DL-alpha	-	0.1% w/w
Propylene glycol	9% w/w	10.5% w/w
Ethanol	10% w/w	10.5% w/w

The microemulsion preconcentrates were prepared as follows: After heating Cremophor® RH40 to 65° C with stirring, the other components were added at the weight ratios indicated. This mixture was stirred for one hour. The clear solution obtained, i.e. the MEPC, was mixed with the test compound and the resulting mixture was stirred at ambient temperature for 8 to 12 hours. Complete dissolution in the MEPC was assessed by crossed polarized light microscopy.

Blood samples (approx. 70 µL) were collected at different time points (typically 15, 30, 60, 120, 180, 240, 360, 480 and 1440 min) from the femoral artery for 24 hours

after oral dosing. After acetonitrile precipitation of blood or plasma samples (25  $\mu$ L), dried residues were re-dissolved in methanol/water, analyzed by HPLC, typically on a C<sub>18</sub> reversed-phase HPLC column. Typical eluents were: 100 % water with 0.1 % HCOOH (pH 2.1), and 100 % acetonitrile with 0.1 % HCOOH. A linear gradient was usually run from 15 to 95 % B over 7 min followed by a 3 min hold at 95 % B at a constant temperature of 40 °C in the column compartment. The flow rate was held constant at 50-100  $\mu$ L/min, as appropriate. Sample injection volume was 10  $\mu$ L. The flow from the HPLC system was directly introduced into the ion source of a triple quadrupole mass analyzer and subjected to atmospheric pressure electrospray ionization. All compounds were detected as protonated quasi-molecular ions [M+H]<sup>+</sup> or appropriate fragments thereof. Structurally closely related compounds were used as analytical internal standards. Quantification of blood or plasma levels of the parent compounds was based on a 7-level calibration curve (in triplicate) using blank rat plasma samples spiked with stock solutions of external and internal standards. Basic pharmacokinetic parameters were estimated using a non-compartmental approach. AUC p.o. was calculated using the trapezoidal rule, C<sub>max</sub> and T<sub>max</sub> taken from the mean blood/plasma level vs. time curve (n=3-4 animals). The C<sub>max</sub> values were dose-normalized.

For selected compounds an additional group of rats received the compound by intravenous injection, and AUC i.v. was determined using an analogous approach as described above for AUC p.o. Absolute oral bioavailability (in %) was then calculated from the dose-normalized AUC p.o. divided by dose-normalized AUC i.v., then multiplied with 100.

9. The compound of claim 13 has an average CaSR FLIPR IC<sub>50</sub> value of 1.35  $\pm$  0.2 nM, as determined by the FLIPR assay recited in paragraph 6 *supra*. Furthermore, the compound of claim 13 exhibits a dose-normalized C<sub>max</sub> of 61.5 nM, a T<sub>max</sub> of 1 hour and a bioavailability of 31% in rat as determined by the PK cassette dosing assay using MEPC-A recited in paragraph 8 *supra*. If dosed as single compound (in a so-called one-in-one experiment), the compound of claim 13 exhibits a dose-normalized C<sub>max</sub> of 54.7 nM, a T<sub>max</sub> of 0.42 hours and a bioavailability of 41% in rat as determined by the PK assay using MEPC-A recited in paragraph 8 *supra*. See Table 1 *infra*.

Table 1 *infra*, displays the average FLIPR CaSR IC<sub>50</sub> value determined by the FLIPR assay recited in paragraph 6 *supra*, and the PK data determined by the PK assay recited in paragraph 8 *supra*, for the compound of claim 13.

Table 1:

claim #	Structure	CaSR FLIPR IC <sub>50</sub> (average) [nM]	C <sub>max</sub> [nM]	T <sub>max</sub> [h]	Bioavailability [%]
claim 13		1.35	61.5 (cassette dosing)	1.0 (cassette dosing)	31 (cassette dosing)
			54.7 (single dosing)	0.42 (single dosing)	41 (single dosing)

10. Table 2 *infra* lists comparative data for exemplified compounds of the '559 application which are structurally related to the compound of claim 13 of the instant application.

The compound of Example 20 carries a 6-methoxy residue instead of the propargyloxy residue at the 6 position, and the N-benzyl is not substituted.

The compound of Example 37 includes the propargyloxy residue at the 6 position but the N-benzyl has been replaced with N-isopropyl.

The compound of Example 80 incorporates both a 6-propargyloxy residue and an N-benzyl group but does not include substitution on the benzyl aromatic ring.

Examples 90 and 120 incorporate a substituted benzyl residue into the structure of Example 80 (e.g., Example 90 includes a para hydroxy and Example 120 includes a para amino).

The compound of Example 148 incorporates an N-(4-chlorobenzyl) residue into a compound which contains a 6-methoxy residue.

The compound of Example 149 incorporates the N-(4-bromobenzyl) residue into a compound which contains a 6-methoxy residue.

The compound of Example 150 incorporates an N-(4-fluorobenzyl) residue into a compound which contains a 6-methoxy residue.

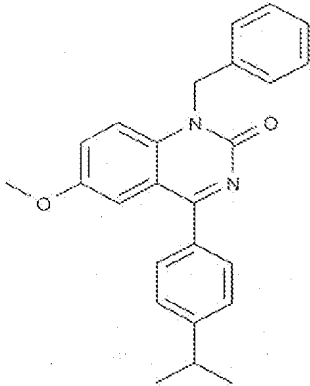
The compound of Example 152 incorporates an N-(4-methoxybenzyl) residue into a compound which contains a 6-methoxy residue.

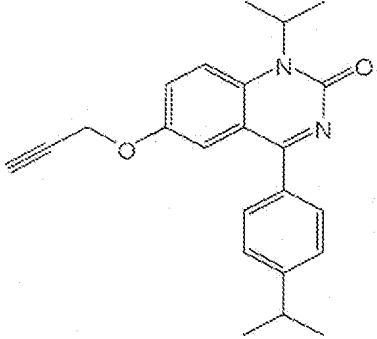
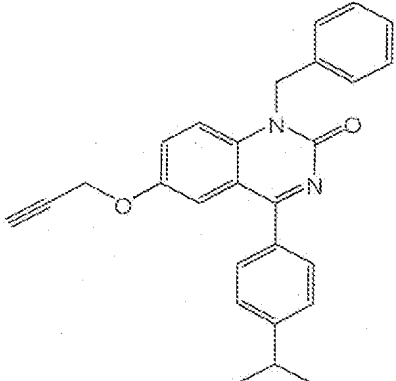
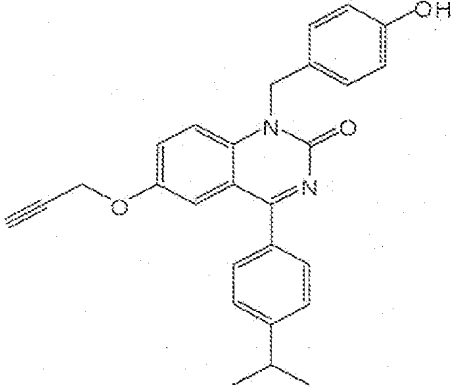
The compound of Example 153 incorporates an N-(4-hydroxybenzyl) residue into a compound which contains a 6-methoxy residue.

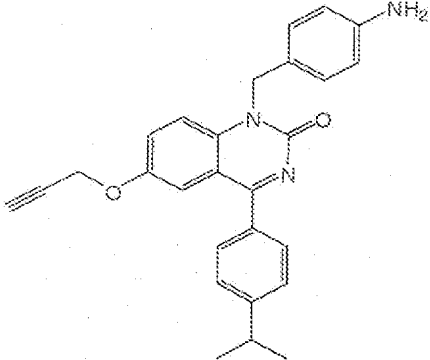
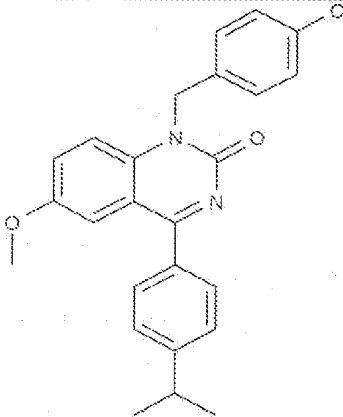
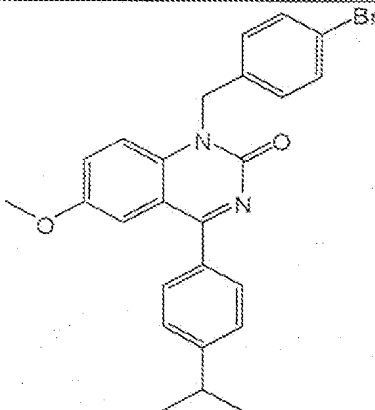
The compound of Example 154 incorporates an N-(4-trifluoromethyl-benzyl) residue into a compound which contains a 6-methoxy residue.

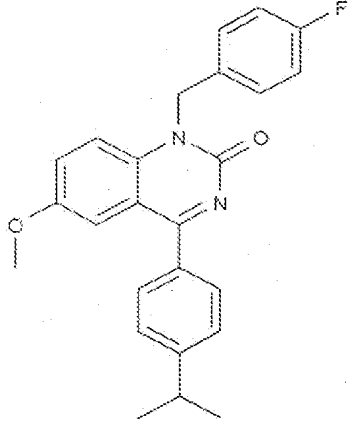
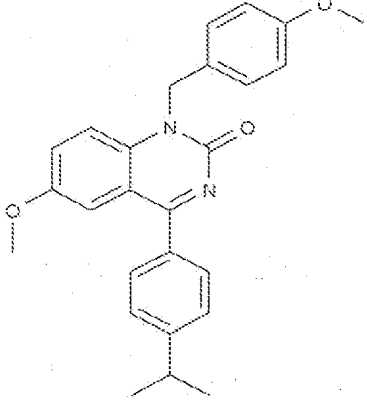
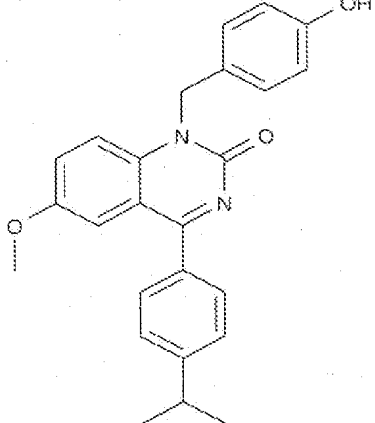
Table 2 *infra*, displays the average PI CaSR  $IC_{50}$  values determined by the PI assay recited in paragraph 7 *supra*, and the average FLIPR CaSR  $IC_{50}$  values determined by the FLIPR assay recited in paragraph 6 *supra*. N.d. refers to not determined.

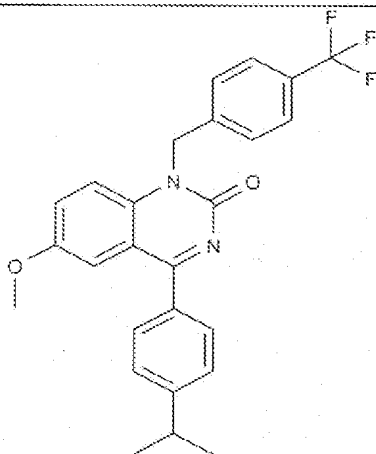
Table 2:

'559 application example #	Structure	PI CaSR $IC_{50}$ [nM]	FLIPR CaSR $IC_{50}$ (average) [nM]
29		160	n.d.

'559 application example #	Structure	PI CaSR IC <sub>50</sub> [nM]	FLIPR CaSR IC <sub>50</sub> (average) [nM]
37		30	8.75
80		5.3	4.4
90		n.d.	10.0

'559 application example #	Structure	PI CaSR IC <sub>50</sub> [nM]	FLIPR CaSR IC <sub>50</sub> (average) [nM]
120		35	15.0
148		130	n.d.
149		150	100

'559 application example #	Structure	PI CaSR IC <sub>50</sub> [nM]	FLIPR CaSR IC <sub>50</sub> (average) [nM]
150		160	290
152		450	n.d.
153		470	n.d.

'559 application example #	Structure	PI CaSR IC <sub>50</sub> [nM]	FLIPR CaSR IC <sub>50</sub> (average) [nM]
154		510	2060

11. For the compounds displayed in Table 2 *supra*, early structure-activity relationship studies were conducted on compounds having a 6-methoxy substituent in lieu of the 6-propargyloxy group (See Examples 29, 148-150 and 152-154). Initial compounds were assessed using the PI assay described in paragraph 7 *supra*. Para-halogen substituted compounds (Examples 148-150) exhibit substantially the same potency in the PI assay as the parent unsubstituted N-benzyl compound (Example 29). Incorporation of other para substituents on the N-benzyl group reduced the observed potency in the PI assay by about 3 fold (See in particular Examples 152-154).

12. From the structurally related compounds of the '559 application listed in Table 2 *supra*, it had been learned that replacement of the 6-methoxy substituent with a 6-propargyloxy group (see Examples 37, 80, 90 and 120) improved the antagonistic potency of the respective compounds at the CaSR by approximately 30 to 50 fold (see Example 29 in comparison to Example 80 and Example 153 in comparison to Example 90). However, there was no general trend deducible concerning the influence of the nature of the N(1) substituent of the 1H-quinazolin-2-ones on activity, since para substitution of the unsubstituted N-benzyl residue with hydroxy or amino resulted in decreased inhibitory activity (see in particular Examples 90 and 120 in comparison to Example 80), whereas replacement of the N-benzyl moiety with an N-isopropyl group

reduced potency (see Example 37). The most active compound in this series was the unsubstituted N-benzyl derivative (Example 80).

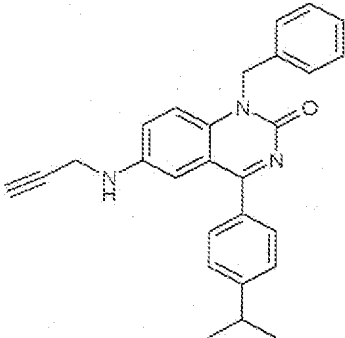
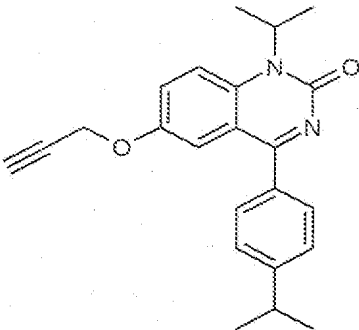
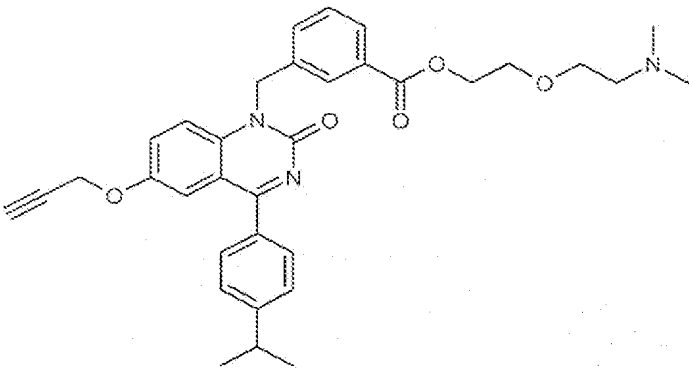
The compound of claim 13 of the instant invention exhibits increased CaSR inhibitory activity (measured with the FLIPR assay) compared to the structurally related compounds of the '559 application listed in Table 2 *supra*. More particularly, the compound of claim 13 of the instant application offers at least a 3 fold improvement in CaSR inhibition in comparison to the unsubstituted N-benzyl derivative (Example 80), an at least a 7 fold improvement in CaSR inhibition in comparison to the other para-substituted N-benzyl derivatives (Examples 90 and 120), and an at least 100 fold improvement in comparison to the 6-methoxy derivative (Example 149).

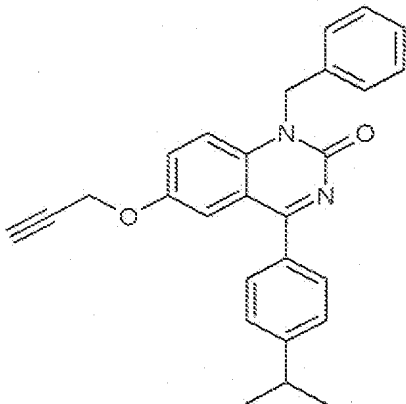
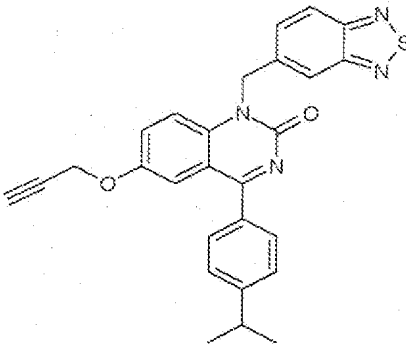
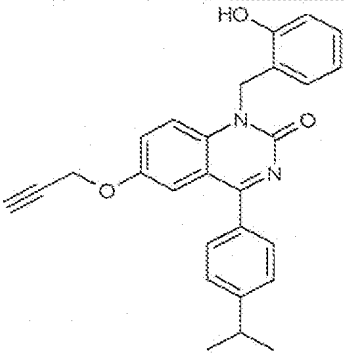
By simply extrapolating the structure-activity relationship between Examples 80, 90, and 120 on the one hand and between Examples 29 and 80 and Examples 29 and 149, respectively on the other hand, and given the huge number of possibilities of variation of the substituents, it is my considered scientific opinion that the '599 application does not disclose, suggest, or even allude to the actually observed improvement in CaSR inhibitory activity by the replacement of the 6-methoxy group in combination with the incorporation of a para-bromo substituent on the N-benzyl residue as provided by the compound of claim 13.

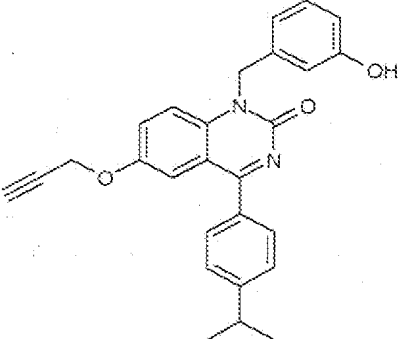
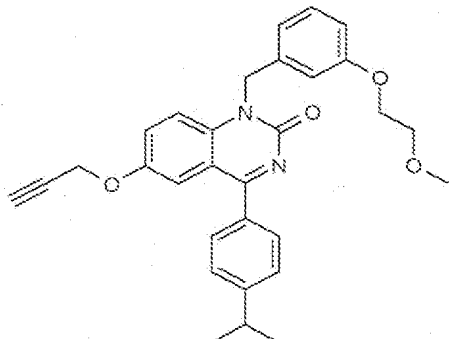
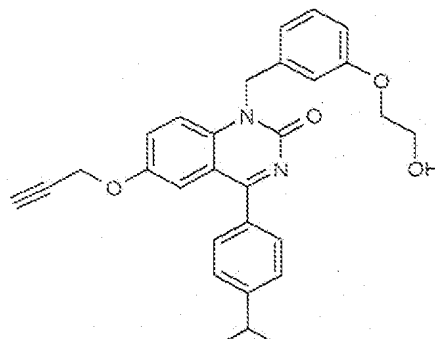
13. The *in vitro* CaSR  $IC_{50}$  data as determined with the FLIPR assay recited in paragraph 6 *supra* for the most potent compounds exemplified in co-pending USSN 10/480,559 are recited in Table 3. Examples 37 and 80 which were included in Table 2 are re-presented in Table 3 for the sake of convenience. Each compound listed in Table 3 exhibits an  $IC_{50}$  of less than 10 nM in the FLIPR assay. None of the compounds in Table 3 include an N-benzyl residue which is substituted at the para position of the N-benzyl ring. The most active exemplified compounds of the '559 application have a meta substituted N-benzyl residue (Examples 89, 106, 110, 116 and 117) or a benzothiadiazolyl-methyl residue (Example 84), each of which has an  $IC_{50}$  of between 2 and 3 nM in the FLIPR assay.

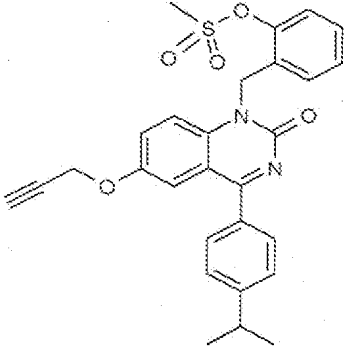
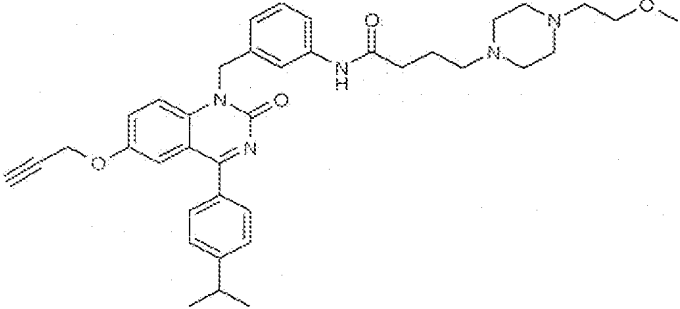
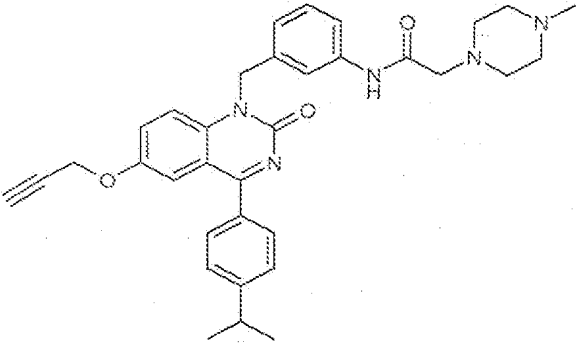
Table 3 *infra*, displays the average FLIPR CaSR  $IC_{50}$  values determined by the FLIPR assay recited in paragraph 6 *supra* for the most potent compounds exemplified in co-pending USSN 10/480,559.

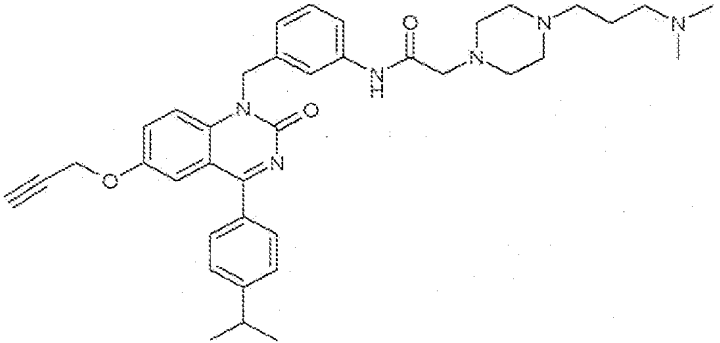
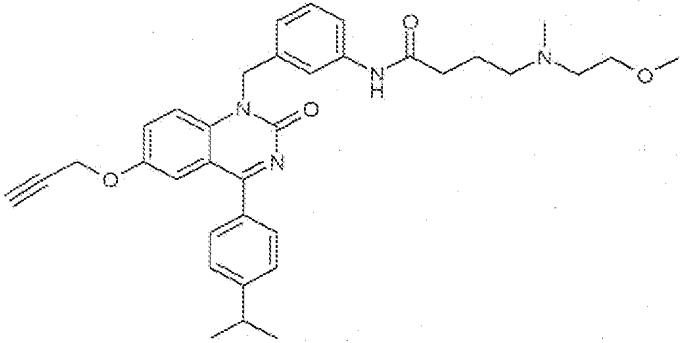
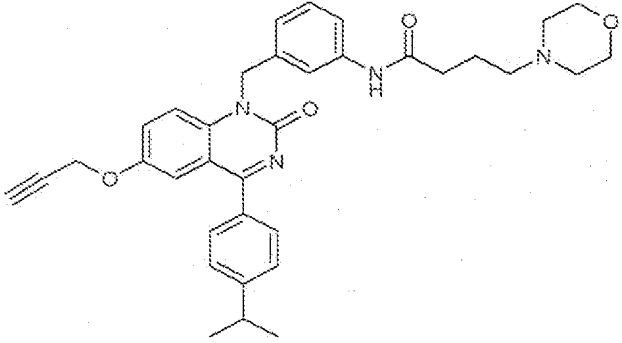
Table 3:

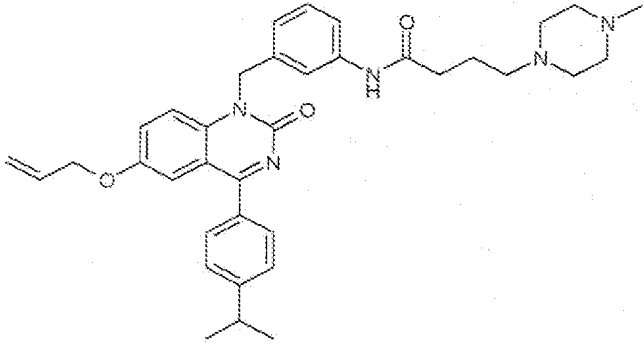
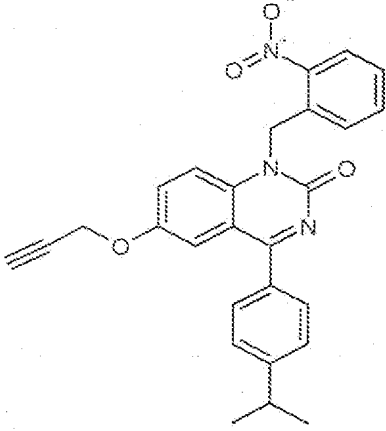
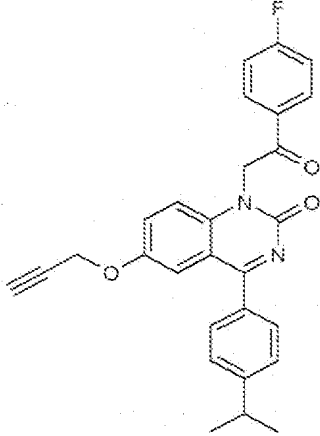
Example Nr.	Formula	FLIPR CaSR IC <sub>50</sub> (average) [nM]
14		6.1
37		8.75
61		4.4

Example Nr.	Formula	FLIPR CaSR IC <sub>50</sub> (average) [nM]
80		4.4
84		2.8
87		4.5

Example Nr.	Formula	FLIPR CaSR IC <sub>50</sub> (average) [nM]
89		2.5
99		9
102		7.3

Example Nr.	Formula	FLIPR CaSR IC <sub>50</sub> (average) [nM]
105		6.2
106		2.8
110		2.4

Example Nr.	Formula	FLIPR CaSR IC <sub>50</sub> (average) [nM]
113		8.1
116		2.9
117		2.5

Example Nr.	Formula	FLIPR CaSR IC <sub>50</sub> (average) [nM]
118		4.6
121		6.4
190		6.3

14. The compound of claim 13 of the instant invention exhibited increased potency in the FLIPR assay, i.e. an increased antagonistic activity at the CaSR, compared with all of the exemplified compounds of the '559 application. More particularly, the compound of claim 13 offers improved potency compared to Example 80, the unsubstituted N-benzyl derivative, of the '559 application and to the most active Examples 110, 117, 89, 84, 106 and 116, carrying either a meta substituted N-benzyl residue (Examples 89, 106, 110, 116 and 117) or a benzothiadiazolyl-N-methyl residue (Example 84).

Extrapolating the structure-activity relationship between the most active Examples 110, 117, 89, 84, 106 and 116, and given the huge number of possibilities of variation of the substituents, it is my considered scientific opinion that the '599 application does not disclose, suggest, or even allude to an improvement in CaSR inhibitory activity by incorporation of a para-bromo substituent instead of one of the exemplified substituents in the meta position on the N-benzyl residue as provided by the compound of claim 13.

15. As pointed out previously, a good calcilytic must not only be a potent antagonist of the CaSR but most importantly its PK profile must be appropriate in order to elicit a bone anabolic effect. The required parameters are high  $C_{max}$  at an early time point ( $T_{max}$ ) and a short terminal half life to ensure expeditious return of exposure and PTH levels to baseline (a sharp peak should be seen at an early time point after administration in the concentration-time graph). As displayed in Table 1 *supra*, the compound of claim 13 of the instant invention exhibited such a significantly improved PK profile as measured by the pharmacokinetic experiments in rats recited in paragraph 8 *supra*. In particular, the compound of claim 13 was shown in the cassette-dosing PK experiment to have a high dose-normalized  $C_{max}$  value of 61.5 nM reached after a short time period ( $T_{max}$  being 1 hour) and a good bioavailability of 31%.

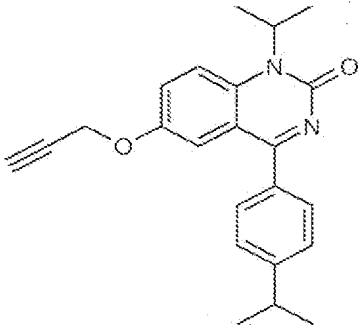
Furthermore, we have found that factor W (quotient of  $C_{max}$  over  $IC_{50}$ ) is a reasonable predictor of "in vivo potency" (provided the PK profile was "calcilytic like", i.e. a sharp peak was seen in the concentration-time graph). The W factor is determined by measuring  $IC_{50}$  data (in human receptor which in the experience of the project team was substantially identical to the rat data) as set out in paragraph 6 or 7 *supra* and the maximum concentration obtained from a rat pharmacokinetic study (the dose normalized  $C_{max}$  values) as set out in paragraph 8 *supra*. A value of factor W of at least 20 was

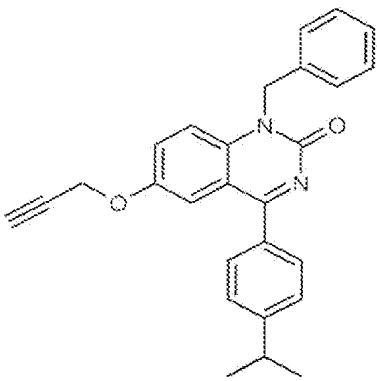
considered necessary to warrant further in vivo investigation for a compound. The compound of claim 13 of the instant invention was predicted to have good "in vivo" potency based on a factor W calculation, since it has a factor W of 45 (i.e.,  $C_{\max}/IC_{50} = 61.5/1.35 = 45$ ).

16. From the compounds of co-pending application USSN 10/480,559 the corresponding pharmacokinetic data were determined for Examples 37 and the structurally closest related Example 80 by the dosing assay recited in paragraph 8 *supra* of co-pending USSN 10/480,559 (Examples 37 was analysed in MECP-B, and Example 80 in MEPC-A) (see Table 4 *infra*).

Table 4 *infra*, displays the average FLIPR CaSR  $IC_{50}$  values determined by the FLIPR assay recited in paragraph 6 *supra*, and the PK data determined by the PK assay recited in paragraph 8 *supra*, for Examples 37 and 80 of co-pending USSN 10/480,559. The factor W was calculated as indicated above (quotient of  $C_{\max}$  over  $IC_{50}$ ).

Table 4

Ex. #	Formula	CaSR FLIPR $IC_{50}$ (average) [nM]	$C_{\max}$ [nM]	$T_{\max}$ [h]	$W = C_{\max} / IC_{50}$ (FLIPR)
37		8.75	37.1	0.21	4.2

Ex. #	Formula	CaSR FLIPR IC <sub>50</sub> (average) [nM]	C <sub>max</sub> [nM]	T <sub>max</sub> [h]	W = C <sub>max</sub> / IC <sub>50</sub> (FLIPR)
80		4.4	23.3	0.42	5.3

17. Of the exemplified compounds of the '559 application having been tested in the PK assay, both compounds, i.e. Examples 80 and 37, demonstrated reasonably high dose normalized C<sub>max</sub> values of over 20 nM, a value which is however lower than the corresponding value for the compound of claim 13. Example 80 and in particular Example 37 both exhibit early T<sub>max</sub> values, i.e. sharp, calcilytic-like PK profiles, but further in vivo pharmacodynamic analysis in rats showed that the compounds exhibited clearly reduced potency in comparison to the compound of claim 13 of the instant application.

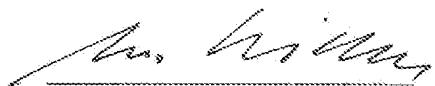
Extrapolating the relationship between structure and pharmacokinetic profile of the analysed calcilytic compounds, and given the huge number of possibilities of variation of the substituents, it is my considered scientific opinion that the '599 application does not disclose, suggest, or even allude to the actually observed pharmacokinetic profile as provided by the compound of claim 13 by incorporation of a para-bromo substituent on the N-benzyl residue.

18. Concerning the "factor W" determined as set out in paragraph 15 *supra*, of the exemplified compounds of the '559 application that have been tested in this regard, both compounds, i.e. Examples 37 and 80, have a factor W below 20 and were indeed shown to have a clearly inferior potency when compared to the compound of claim 13 of the instant application as already set out in paragraph 17 *supra*.

19. In summary, the compound of claim 13 exhibits a  $C_{max}$  of 61.5 nM, a  $T_{max}$  of 1 hour and a bioavailability of 31% in rat. Moreover the W factor for the compound of claim 13 is 45. Accordingly, the compound of claim 13 provides excellent potency, a good PK profile (characterized by  $C_{max}$ ,  $T_{max}$  and bioavailability) and a factor W value ( $C_{max}$  to  $IC_{50}$  ratio) predictive of a good *in vivo* potency.

20. As set out in detail above, the superior *in vitro* and *in vivo* potency provided by the compound of claim 13 of the instant invention is not found in, nor is it suggested by the examples of the '559 application.

21. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



Dr. Leo Widler

Oct 13, 2009

Date

Appendix A

Curriculum Vitae

Dr. Leo Widler

August 2009

Curriculum Vitae

**Dr. Leo Widler**

Senior Research Investigator I  
Novartis Research Institutes for BioMedical Research, Basel, Switzerland

1975 - 1979	Undergraduate Studies in chemistry at the Federal Institute of Technology (ETH), Zurich Switzerland
1979	M.S. thesis with Prof. A. Eschenmoser Federal Institute of Technology (ETH), Zurich Switzerland
1980 - 1983	PhD thesis with Prof. D. Seebach Federal Institute of Technology (ETH), Zurich <i>Title: Regio- and diastereoselective reactions of allyl-titanium compounds</i>
1983 - 1985	Post-doc at the Sandoz Research Institute E. Hanover N.J. / USA working on HMG-CoA reductase inhibitors (Lescol®)
1985 - 1994	Labhead Pharma Division Ciba-Geigy, Basle Section Inflammation, Bone & Allergy working on antirheumatics and bisphosphonates (Zometa® / Acclasta®)
Jan. - March 88	Job rotation Hindustan Ciba Geigy Research Institute Goregaon, Mumbai (Bombay) / INDIA
1994 - 1995	Job rotation Ciba Summit N.J. / USA Arthritis Unit working on COX-2 inhibitors (Prexige®)
1995 - 1996	CIBA Basel / Switzerland Labhead in the Bone Metabolism Group of the Chemistry Resources Unit and Oncology working on c-Src inhibitors
1997 - 1999	Labhead Bone Metabolism Unit, Novartis
2000 - present	Project Team Leader Bone Metabolism Unit and Global Discovery Chemistry responsible for the Calcilytics/PTH release project